# RESPIRATION AFTER DEATH[1]

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(WITH THREE FIGURES)

It is commonly stated that when respiration ceases the protoplasm is no longer alive, but it is uncertain in most cases whether respiration ends as soon as death occurs or whether it continues for some time afterward.

It was stated by JOHANNSEN (8), by DETMER (6), and by PFEFFER (17) that in general there is no production of $CO_2$ after death, although REINKE (18) and BRENSTEIN (3) held the opposite view. BUCHNER (4) showed that yeast which had been treated with acetone and ether and which was incapable of cell division, and in all probability dead, could produce $CO_2$ by fermentation. KOSTYTSCHEFF (10) found that an aërobic plant, *Aspergillus niger*, treated in this manner was still capable of respiration. Since some of the cells appeared to be alive after the treatment, he used heat to kill them. After this the oxidation was extremely small. This is to be expected as the oxidases are, for the most part, injured or destroyed by heat. Similar experiments have been made on bacteria. WARBURG (21) obtained a completely sterile acetone preparation of *Staphylococcus* which respired about one thirty-sixth as much as the living material. WARBURG and MEYERHOF (21) found that treatment with acetone and ether had little effect on the consumption of oxygen by unfertilized sea urchin eggs (although they were completely killed), but the same treatment diminished the consumption of oxygen by fertilized eggs by 90 per cent.

Numerous experiments have been made with cells killed by mechanical means (finely ground) or by freezing and thawing. PALLADIN (16) found that finely ground wheat produced less $CO_2$ than the normal amount, while various plants exposed to $-20°$ C. for some time and then thawed out showed a loss of power to absorb oxygen, but continued to produce $CO_2$.

[1] A preliminary communication appeared in Proc. Nat. Acad. Sci.

Batelli and Stern (2) have found oxidation in finely ground tissue and watery extracts. Their results have been criticized by Warburg.

Warburg (21) found that the finely ground red blood corpuscles of birds consumed less oxygen than intact cells. Unfertilized sea urchin eggs, cytolyzed in distilled water, consumed as much oxygen as the intact eggs but produced no $CO_2$. In fertilized eggs cytolysis reduced the oxygen consumption by 90 per cent or more. A fuller account of the literature seems unnecessary, as it has been summarized by Warburg.

It will be noticed that in the cases previously reported respiration after death is greatly reduced or entirely lacking. The only instance in which post mortem respiration is greater than in normal tissue is that reported by Loeb and Wasteneys (11), in which unfertilized sea urchin eggs, cytolyzed by saponin, showed from 3 to 7 times the normal rate of respiration. It is of considerable interest therefore to find that the respiration of *Laminaria* after death may be much greater than when in its normal condition.

The determination of the output of $CO_2$ was made in the following manner. The increase in the hydrogen ion concentration of sea water containing pieces of *Laminaria* (in the dark) served as a measure of the respiration of the tissue. The decrease in PH value was determined by the addition of a suitable indicator (phenolsulphone phthalein) by comparing the colors with those of a series of buffer mixtures containing an equal amount of the same indicator.

Each piece of *Laminaria* was kept for about half an hour in sea water before beginning the experiment. This treatment tended to obviate any effects of the slight wounding (19, 20). The material was then rolled into a scroll and inserted into a Pyrex glass tube (7) fused shut at one end and attached to a paraffined rubber tube at the open end. Sea water, of the temperature of the bath ($16 \pm 1$°C.), was placed in the tube and the latter inserted in a black enameled collapsible tin tube in the bath. The sea water surrounding the tissue (in the tube) was renewed several times before beginning the experiment. A definite amount of sea water

(6 cc.) was placed in each of the tubes. The tubes were clamped shut in such a way as to include a very small air bubble (always of the same size) which served as a stirrer. This was sufficiently accurate and was more convenient than paraffined glass beads. After a tube had been in the dark at 16± 1°C. for a definite period, it was removed from the bath, and the contents shaken by inverting the tube several times. The sea water was then poured rapidly into an empty tube of equal diameter, to which the same quantity (3 drops of 0.01 per cent aqueous phenolsulphone phthalein to 6 cc. of solution) of indicator was added as had been added to the buffer mixtures. The color was then compared with the colors of a series of buffer mixtures by the use of a constant source of light (the "Daylight" lamp) and the PH value determined. The same amount of sea water was again added to the tissue in the tube and the tube exposed (at 16± 1°C. in the dark) for the same length of time as before, after which it was removed from the bath and the PH value again determined. This was repeated until the respiration in sea water was approximately constant. Then sea water containing the killing agent was substituted for the sea water, and the PH values determined as before after a series of successive periods (each of the same length as the original).

In some cases (acetone 17.4 and alcohol 24.2 per cent) the killing agent extracted from the plant a small amount of pigment which interfered with the color of the indicator.[2] This difficulty disappeared after the first two periods, however, as was shown by running pure hydrogen through the solution, after which it returned to the color found in normal sea water containing indicator. This method also showed conclusively that the only acid excreted by the plant was carbonic acid.

The methods of killing the tissue were various. Sea water containing anesthetics (made up to the conductivity of sea water by the addition of concentrated sea water) was employed in many of the experiments. In this case the respiration was determined for several periods of equal length in sea water (the solution being renewed after each period). The sea water was then replaced by

[2] This did not occur with low concentrations of these substances.

sea water containing anesthetic and the respiration determined after successive equal periods until death ensued, and for some time thereafter.

TABLE I

CONTROL FOR I A: 7 PERIODS (27.5 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

| Period | Change in PH | Total change in PH=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration =A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1........ | 8.1−7.6 =0.5 | 0.5 | 0.5 | 1.00 | 27.5 | 0.5 ÷0.5=1.00 |
| 2........ | 8.1−7.6 =0.5 | 1.0 | 1.0 | 1.00 | 55.0 | 0.5 ÷0.5=1.00 |
| 3........ | 8.1−7.6 =0.5 | 1.5 | 1.5 | 1.00 | 82.5 | 0.5 ÷0.5=1.00 |
| 4........ | 8.1−7.6 =0.5 | 2.0 | 2.0 | 1.00 | 110.0 | 0.5 ÷0.5=1.00 |
| 5........ | 8.1−7.65=0.45 | 2.45 | 2.5 | 0.98 | 137.5 | 0.45÷0.5=0.90 |
| 6........ | 8.1−7.65=0.45 | 2.90 | 3.0 | 0.96 | 165.0 | 0.45÷0.5=0.90 |
| 7........ | 8.1−7.65=0.45 | 3.35 | 3.5 | 0.96 | 192.5 | 0.45÷0.5=0.90 |

TABLE I A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (22 MIN. EACH) IN SEA WATER AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

| Solution | Period | Change in PH | Change in PH during saturation with ethyl bromide=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration=A/B | Time in min. | Time during saturation with ethyl bromide in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|---|---|
| Sea water.......... | 1 | 8.1 −7.8 =0.30 | ...... | ...... | ...... | 22 | 0 | ............... |
| " " .......... | 2 | 8.1 −7.8 =0.30 | ...... | ...... | ...... | 44 | 0 | ............... |
| Sea water containing ethyl bromide..... | 3 | 7.32−6.16*=1.16+ | 1.16 | 0.30 | 3.8 | 66 | 22 | 1.16÷0.30=3.8 |
| Sea water containing ethyl bromide..... | 4 | 7.32−6.16*=1.16+ | 2.32 | 0.60 | 3.8 | 88 | 44 | 1.16÷0.30=3.8 |
| Sea water containing ethyl bromide..... | 5 | 7.32−6.69 =0.63 | 2.95 | 0.90 | 3.2 | 110 | 66 | 0.63÷0.30=2.1 |
| Sea water containing ethyl bromide..... | 6 | 7.32−7.07 =0.25 | 3.20 | 1.20 | 2.6 | 132 | 88 | 0.25÷0.30=0.8 |
| Sea water containing ethyl bromide..... | 7 | 7.32−7.21 =0.11 | 3.31 | 1.50 | 2.2 | 154 | 110 | 0.11÷0.30=0.4 |
| Sea water containing ethyl bromide..... | 8 | 7.32−7.32 =0.0 | 3.31 | 1.80 | 1.8 | 176 | 132 | 0.0 ÷0.30=0.0 |

* Approximate (at this point indicator is not very sensitive to slight changes in acidity).

As it was important to know the time of death as accurately as possible, determinations of the electrical conductivity of the tissue were made by the method of OSTERHOUT (12, 13). If the electrical resistance of the normal tissue be called 100 per cent, it is found that after killing the resistance falls to about 10 per cent. When the resistance has fallen to 15 per cent the tissue is for all practical purposes dead, as there is no recovery when it is returned to normal conditions.[3]

The results of the experiments showing the relative amount and relative rate of respiration of tissue of *Laminaria* when subjected to various treatments are presented in tabular form. In every case 6–12 or more closely agreeing results were obtained and the data

TABLE I B

Net electrical resistance of *Laminaria* in sea water and in sea water approximately saturated with ethyl bromide, expressed as percentage of net resistance at start of experiment at 20° C.

| Sea water | | Sea water approximately saturated with ethyl bromide | |
|---|---|---|---|
| Time in min. | Percentage net resistance | Time in min. | Percentage net resistance |
| 0 | 100 | 0 | 100 |
| 100 | 90.9 | 1 | 105 |
| 200 | 87.8 | 5 | 79 |
| | | 10 | 56 |
| | | 15 | 39 |
| | | 20 | 30 |
| | | 35 | 16 |
| | | 60 | 11 |
| | | 80 | 10 |
| | | 1000 | 5 |

of a typical case presented. Table I A shows the relative amount and relative rate of respiration as influenced by sea water approximately saturated with ethyl bromide. This is the only experiment in which the PH value of the control differed from the PH value of the sea water containing anesthetic (at the start of the experiment). It will be noted that after about 130 minutes no respiration was detected. An examination of table I B, which gives the net electrical resistance of *Laminaria*, shows that the material can be considered dead before the end of 60 minutes. From table I A it is seen that at the end of 60 minutes the relative rate and relative

[3] The determinations were made in part by Professor Osterhout and in part by me.

amount of respiration are approximately double that of the normal, although the tissue is shown by the method of electrical resistance to be dead. This is brought out very strikingly in fig. 1 where we plot as ordinates the relative amount of respiration (curve C, table I A), relative rate of respiration (curve B, table I A), net resistance as percentage of that at the start (curve A, table I B) respectively (unbroken lines). When the relative rate of respiration has practically reached zero (curve B) the relative amount of

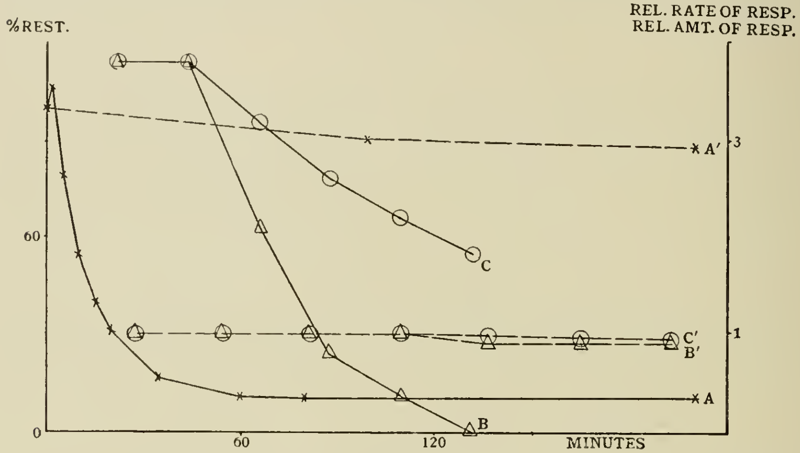


FIG. 1.—Curves showing effect produced by sea water, approximately saturated with ethyl bromide, upon relative amount and relative rate of respiration, and upon net electrical resistance of *Laminaria:* curve A, ordinates represent net resistance as percentage of that at start; curve B, ordinates represent relative rate of respiration; curve C, ordinates represent relative amount of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

respiration is above unity. At the end of 60 minutes, when the tissue can be considered dead, the relative rate is seen to be about double that of the normal rate.

Table II A shows the effect produced by sea water containing 17.4 per cent (by volume) acetone, made up to the electrical conductivity of sea water by the addition of concentrated sea water, upon the relative amount and relative rate of respiration of *Laminaria*. At the end of 2.5 hours the rate of respiration is still above the normal rate, while the relative amount of respiration is nearly 2. Table II B shows the net electrical resistance. It is seen that the material is dead before the end of 100 minutes.

TABLE II

CONTROL FOR II A: 9 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

| Period | Change in PH | Total change in PH=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration =A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1....... | 8.37−7.67=0.70 | 0.70 | 0.70 | 1.0 | 21 | 0.70÷0.70=1.0 |
| 2....... | 8.37−7.67=0.70 | 1.40 | 1.40 | 1.0 | 42 | 0.70÷0.70=1.0 |
| 3....... | 8.37−7.70=0.67 | 2.07 | 2.10 | 0.99 | 63 | 0.67÷0.70=0.95 |
| 4....... | 8.37−7.73=0.64 | 2.71 | 2.80 | 0.97 | 84 | 0.64÷0.70=0.91 |
| 5....... | 8.37−7.73=0.64 | 3.35 | 3.50 | 0.96 | 105 | 0.64÷0.70=0.91 |
| 6....... | 8.37−7.73=0.64 | 3.99 | 4.20 | 0.95 | 126 | 0.64÷0.70=0.91 |
| 7....... | 8.37−7.72=0.65 | 4.64 | 4.90 | 0.95 | 147 | 0.65÷0.70=0.93 |
| 8....... | 8.37−7.73=0.64 | 5.28 | 5.60 | 0.94 | 168 | 0.64÷0.70=0.91 |
| 9....... | 8.37−7.72=0.65 | 5.93 | 6.30 | 0.94 | 189 | 0.65÷0.70=0.93 |

TABLE II A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (24 MIN. EACH) IN SEA WATER, AND DURING 7 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

| Solution | Period | Change in PH | Change in PH during exposure to acetone=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration=A/B | Time in min. | Time of exposure to acetone in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|---|---|
| Sea water............ | 1 | 8.37−7.76=0.61 | ...... | ...... | ...... | 24 | 0 | ............... |
| " " ............ | 2 | 8.37−7.78=0.59 | ...... | ...... | ...... | 48 | 0 | ............... |
| Sea water containing 17.4 per cent acetone | 3 | 8.37−6.80=1.57 | 1.57 | 0.60 | 2.61 | 72 | 24 | 1.57÷0.60=2.6 |
| Sea water containing 17.4 per cent acetone | 4 | 8.37−6.75=1.62 | 3.19 | 1.20 | 2.66 | 96 | 48 | 1.62÷0.60=2.7 |
| Sea water containing 17.4 per cent acetone | 5 | 8.37−7.06=1.31 | 4.50 | 1.80 | 2.50 | 120 | 72 | 1.31÷0.60=2.2 |
| Sea water containing 17.4 per cent acetone | 6 | 8.37−7.50=0.87 | 5.37 | 2.40 | 2.24 | 144 | 96 | 0.87÷0.60=1.5 |
| Sea water containing 17.4 per cent acetone | 7 | 8.37−7.54=0.83 | 6.20 | 3.00 | 2.07 | 168 | 120 | 0.83÷0.60=1.4 |
| Sea water containing 17.4 per cent acetone | 8 | 8.37−7.62=0.75 | 6.95 | 3.60 | 1.93 | 192 | 144 | 0.75÷0.60=1.3 |
| Sea water containing 17.4 per cent acetone | 9 | 8.37−7.74=0.63 | 7.58 | 4.20 | 1.80 | 216 | 168 | 0.63÷0.60=1.1 |

The fact that respiration proceeds here at a rate much above the normal (although death has taken place) is very clearly brought out by comparing the curves for table II A and II B as given in fig. 2. The ordinates represent relative amount of respiration (curve A, table II A), relative rate of respiration (curve C, table II A), net resistance as percentage of that at the start (curve B, table II B) respectively (unbroken lines). The relative rate and relative amount of respiration at the end of over 2.5 hours are still much above the normal even though the measurements of electrical resistance have shown the tissue to be dead before 100 minutes.


FIG. 2.—Curves showing effect produced by sea water containing 17.4 per cent (by volume) of acetone upon relative amount and relative rate of respiration, and effect produced by sea water containing 16.2 per cent of acetone upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

Table III A shows the effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol (made up to conductivity of sea water by the addition of concentrated sea water). In fig. 3 the ordinates represent: relative amount of respiration (curve A, table III A), relative rate of respiration (curve C, table III A), net resistance as percentage of that at the start (curve B, table III B) respectively (unbroken lines). If we consider the material dead at the end of 90 minutes, we find that the rate of respiration is much above the normal rate, while the relative amount of respiration is above 2.

TABLE II B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 16.2 PER CENT (BY VOLUME) OF ACETONE, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 15.4° C.

| Sea water | | Sea water containing 16.2 per cent acetone | |
|---|---|---|---|
| Time in min. | Percentage net resistance | Time in min. | Percentage net resistance |
| 0 | 100 | 0 | 100 |
| 100 | 90.9 | 5 | 105.5 |
| 200 | 87.8 | 10 | 94.3 |
| | | 20 | 46.6 |
| | | 30 | 28.4 |
| | | 40 | 22.7 |
| | | 50 | 20.2 |
| | | 60 | 18.2 |
| | | 70 | 16.2 |
| | | 80 | 13.2 |
| | | 100 | 11.1 |
| | | 200 | 8.7 |
| | | 300 | 6.7 |

TABLE III

CONTROL FOR III A: 8 PERIODS (30.25 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

| Period | Change in PH | Total change in PH=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration =A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1 | 7.90−7.53=0.37 | 0.37 | 0.37 | 1.0 | 30.25 | 0.37÷0.37=1.0 |
| 2 | 7.90−7.53=0.37 | 0.74 | 0.74 | 1.0 | 60.50 | 0.37÷0.37=1.0 |
| 3 | 7.90−7.53=0.37 | 1.11 | 1.11 | 1.0 | 90.75 | 0.37÷0.37=1.0 |
| 4 | 7.90−7.54=0.36 | 1.47 | 1.48 | 0.99 | 121.00 | 0.36÷0.37=0.97 |
| 5 | 7.90−7.54=0.36 | 1.83 | 1.85 | 0.99 | 151.25 | 0.36÷0.37=0.97 |
| 6 | 7.90−7.55=0.35 | 2.18 | 2.22 | 0.98 | 181.50 | 0.35÷0.37=0.95 |
| 7 | 7.90−7.55=0.35 | 2.53 | 2.59 | 0.97 | 211.75 | 0.35÷0.37=0.95 |
| 8 | 7.90−7.58=0.32 | 2.85 | 2.96 | 0.96 | 242.00 | 0.32÷0.37=0.87 |

Table IV shows the effect produced upon the relative amount and relative rate of respiration of *Laminaria* by sea water containing 3.2 per cent (by volume) of formaldehyde. The solution was made up to the conductivity of sea water by the addition of concentrated sea water. The free acid of the formaldehyde was first neutralized by the addition of a little sodium carbonate. This is allowable for the purposes of the present investigation, for its effect would be to make the amount of $CO_2$ produced appear somewhat less than was actually the case. At the end of 4 hours the relative rate of respiration was still above the normal, while the

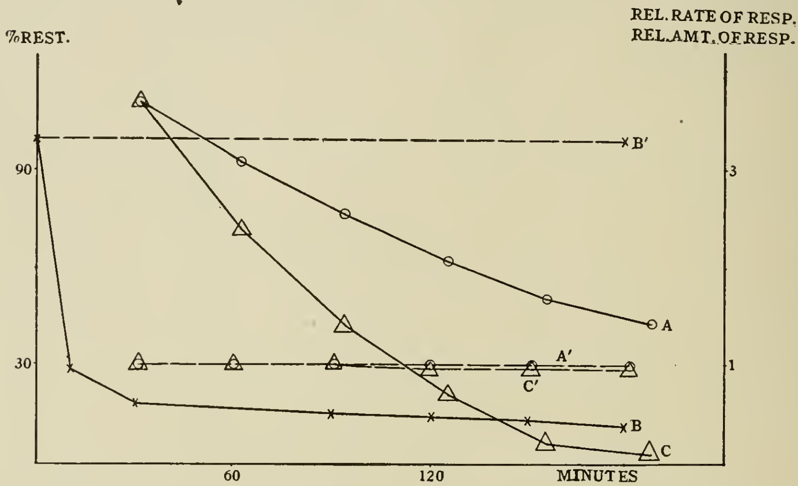


FIG. 3.—Curves showing effect produced by sea water containing 24.2 per cent (by volume) of ethyl alcohol upon relative amount and relative rate of respiration and upon net electrical resistance of *Laminaria:* curve A, ordinates represent relative amount of respiration; curve B, ordinates represent net resistance as percentage of that at start; curve C, ordinates represent relative rate of respiration (unbroken lines); controls in sea water (broken lines); each control curve bears same symbol and letter (with a prime) as experimental curve; abscissae represent time in minutes.

relative amount of respiration was much above the normal. At this concentration of formaldehyde *Laminaria* is practically dead in 180 minutes. In table IV, however, after 280 minutes the relative rate of respiration of *Laminaria* is still above normal, while at 180 minutes the relative rate is far above normal.

For purposes of comparison other methods of killing were tried. By making preliminary conductivity experiments with *Laminaria*, it was found that when it is dried upon cheesecloth in the sunlight in a current of dry air, we can consider the tissue practically dead in 135 minutes. After such treatment the material becomes green. It was placed in sea water for 14 minutes; it lost its crispness and

TABLE III A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 2 PERIODS (31.25 MIN. EACH) IN SEA WATER AND DURING 6 SUBSEQUENT PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

| Solution | Period | Change in PH | Change in PH during the 6 later periods = A | Change in PH calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Time (in min.) of exposure during the 6 later periods | Relative rate of respiration |
|---|---|---|---|---|---|---|---|---|
| Sea water | 1 | 7.90−7.43 =0.47 | ...... | ...... | ...... | 31.25 | 0 | ............... |
| " " | 2 | 7.90−7.43 =0.47 | ...... | ...... | ...... | 62.50 | 0 | ............... |
| Sea water containing 24.2 per cent ethyl alcohol | 3 | 7.90−6.16*=1.74 | 1.74 | 0.47 | 3.57 | 93.75 | 31.25 | 1.74÷0.47=3.7 |
| Sea water containing 24.2 per cent ethyl alcohol | 4 | 7.90−6.75 =1.15 | 2.89 | 0.94 | 3.07 | 125.00 | 62.50 | 1.15÷0.47=2.4 |
| Sea water containing 24.2 per cent ethyl alcohol | 5 | 7.90−7.25 =0.65 | 3.54 | 1.41 | 2.51 | 156.25 | 93.75 | 0.65÷0.47=1.4 |
| Sea water containing 24.2 per cent ethyl alcohol | 6 | 7.90−7.55 =0.35 | 3.89 | 1.88 | 2.07 | 187.50 | 125.00 | 0.35÷0.47=0.7 |
| Sea water containing 24.2 per cent ethyl alcohol | 7 | 7.90−7.80 =0.10 | 3.99 | 2.35 | 1.69 | 218.75 | 156.25 | 0.10÷0.47=0.2 |
| Sea water containing 24.2 per cent ethyl alcohol | 8 | 7.90−7.84 =0.06 | 4.05 | 2.82 | 1.43 | 250.00 | 187.50 | 0.06÷0.47=0.1 |

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE III B

NET ELECTRICAL RESISTANCE OF *Laminaria* IN SEA WATER AND IN SEA WATER CONTAINING 24 PER CENT (BY VOLUME) OF ETHYL ALCOHOL, EXPRESSED AS PERCENTAGE OF NET RESISTANCE AT START OF EXPERIMENT AT 13.3° C.

| Sea water | | Sea water containing 24 per cent ethyl alcohol | |
|---|---|---|---|
| Time in min. | Percentage net resistance | Time in min. | Percentage net resistance |
| 0 | 100 | 0 | 100 |
| 240 | 98 | 10 | 28.8 |
| | | 30 | 18.6 |
| | | 90 | 15.2 |
| | | 120 | 14.9 |
| | | 150 | 13.5 |
| | | 180 | 11.8 |
| | | 210 | 11.3 |

became flaccid. The relative amount and relative rate of respiration of pieces of *Laminaria* were determined before and after the drying treatment (which killed the tissue). The results are given in table V A, the control data being given in table V. The relative amount of respiration after the treatment was very high and after 2 hours was still at 3. The relative rate of respiration after the treatment was very high at the start but gradually declined to normal in 2 hours.

TABLE IV

CONTROL FOR IV A: 15 PERIODS (21 MIN. EACH) IN SEA WATER; SOLUTION RENEWED AT BEGINNING OF EACH PERIOD

| Period | Change in PH | Total change in PH=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration =A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1...... | 8.37−7.80=0.57 | 0.57 | 0.52 | 1.1 | 21 | 0.57÷0.52=1.10 |
| 2...... | 8.37−7.90=0.47 | 1.04 | 1.04 | 1.0 | 42 | 0.47÷0.52=0.90 |
| 3...... | 8.37−7.90=0.47 | 1.51 | 1.56 | 0.96 | 63 | 0.47÷0.52=0.90 |
| 4...... | 8.37−7.90=0.47 | 1.98 | 2.08 | 0.95 | 84 | 0.47÷0.52=0.90 |
| 5...... | 8.37−7.92=0.45 | 2.43 | 2.60 | 0.93 | 105 | 0.45÷0.52=0.87 |
| 6...... | 8.37−7.92=0.45 | 2.88 | 3.12 | 0.92 | 126 | 0.45÷0.52=0.87 |
| 7...... | 8.37−7.92=0.45 | 3.33 | 3.64 | 0.92 | 147 | 0.45÷0.52=0.87 |
| 8...... | 8.37−7.92=0.45 | 3.78 | 4.16 | 0.90 | 168 | 0.45÷0.52=0.87 |
| 9...... | 8.37−7.90=0.47 | 4.25 | 4.68 | 0.90 | 189 | 0.47÷0.52=0.90 |
| 10...... | 8.37−7.90=0.47 | 4.72 | 5.20 | 0.91 | 210 | 0.47÷0.52=0.90 |
| 11...... | 8.27−7.90=0.47 | 5.19 | 5.72 | 0.91 | 231 | 0.47÷0.52=0.90 |
| 12...... | 8.37−7.90=0.47 | 5.66 | 6.24 | 0.91 | 252 | 0.47÷0.52=0.90 |
| 13...... | 8.37−7.90=0.47 | 6.13 | 6.76 | 0.91 | 273 | 0.47÷0.52=0.90 |
| 14...... | 8.37−7.90=0.47 | 6.60 | 7.28 | 0.91 | 294 | 0.47÷0.52=0.90 |
| 15...... | 8.37−7.90=0.47 | 7.07 | 7.80 | 0.91 | 315 | 0.47÷0.52=0.90 |

Another method used in killing the tissue was by placing it in running tap water. A preliminary determination of the electrical resistance showed that 22 hours were more than sufficient to kill the tissue. The experiment was begun at 11:18 A.M. and at 7:50 P.M. the tissue was still somewhat alive, but at 9:25 A.M. next day the tissue had in all probability been dead for some time. The respiration was then determined before and after exposure to running tap water for 19 hours. The results are given in table VI, the data for the control being given in table V A. In table VI it is obvious that no respiration of the tissue was observable after it had been in tap water for 19 hours. There is of course the possibility that the rise and decline of the respiration after death was so rapid as to escape observation if the tissue had been dead much before the end of 19 hours.

TABLE IV A

Change in PH value of sea water produced by respiration of *Laminaria* during 2 periods (23.5 min. each) in sea water and during 13 subsequent periods in sea water containing 3.2 per cent formaldehyde

| Solution | Period | Change in PH | Change in PH during exposure to formaldehyde = A | Change in PH calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Time of exposure to formaldehyde in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|---|---|
| Sea water | 1 | 8.37−7.82=0.55 | ...... | ...... | ...... | 23.5 | 0 | ................ |
| " " | 2 | 8.37−7.82=0.55 | ...... | ...... | ...... | 47.0 | 0 | ................ |
| Sea water containing 3.2 per cent formaldehyde | 3 | 8.37−6.90=1.47 | 1.47 | 0.55 | 2.67 | 70.5 | 23.5 | 1.47÷0.55=2.7 |
| Sea water containing 3.2 per cent formaldehyde | 4 | 8.37−6.90=1.47 | 2.94 | 1.10 | 2.67 | 94.0 | 47.0 | 1.47÷0.55=2.7 |
| Sea water containing 3.2 per cent formaldehyde | 5 | 8.37−7.05=1.32 | 4.26 | 1.65 | 2.58 | 117.5 | 70.5 | 1.32÷0.55=2.4 |
| Sea water containing 3.2 per cent formaldehyde | 6 | 8.37−7.15=1.22 | 5.48 | 2.20 | 2.49 | 141.0 | 94.0 | 1.22÷0.55=2.2 |
| Sea water containing 3.2 per cent formaldehyde | 7 | 8.37−7.33−1.04 | 6.52 | 2.75 | 2.37 | 164.5 | 117.5 | 1.04÷0.55=1.9 |
| Sea water containing 3.2 per cent formaldehyde | 8 | 8.37−7.50=0.87 | 7.39 | 3.30 | 2.24 | 188.0 | 141.0 | 0.87÷0.55=1.6 |
| Sea water containing 3.2 per cent formaldehyde | 9 | 8.37−7.55=0.82 | 8.21 | 3.85 | 2.13 | 211.5 | 164.5 | 0.82÷0.55=1.5 |
| Sea water containing 3.2 per cent formaldehyde | 10 | 8.37−7.58=0.79 | 9.00 | 4.40 | 2.04 | 235.0 | 188.0 | 0.79÷0.55=1.4 |
| Sea water containing 3.2 per cent formaldehyde | 11 | 8.37−7.60=0.77 | 9.77 | 4.95 | 1.97 | 258.5 | 211.5 | 0.77÷0.55=1.4 |
| Sea water containing 3.2 per cent formaldehyde | 12 | 8.37−7.65=0.72 | 10.49 | 5.50 | 1.90 | 282.0 | 235.0 | 0.72÷0.55=1.3 |
| Sea water containing 3.2 per cent formaldehyde | 13 | 8.37−7.70=0.67 | 11.16 | 6.05 | 1.84 | 305.5 | 258.5 | 0.67÷0.55=1.2 |
| Sea water containing 3.2 per cent formaldehyde | 14 | 8.37−7.80=0.57 | 11.73 | 6.60 | 1.77 | 329.0 | 282.0 | 0.57÷0.55=1.0 |
| Sea water containing 3.2 per cent formaldehyde | 15 | 8.37−7.85=0.52 | 12.25 | 7.15 | 1.71 | 352.5 | 305.5 | 0.52÷0.55=0.95 |

TABLE V

Control for V A: 3 periods (25.75 min. each) in sea water; between second and third periods an interval of 19 hours during which tissue was bathed in running sea water

| Period | Change in PH | Total change in PH=A | Change in PH calculated from first 2 periods=B | Relative amount of respiration =A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1....... | 8.0−7.58=0.42 | 0.42 | 0.42 | 1.00 | 25.75 | 0.42÷0.42=1.00 |
| 2....... | 8.0−7.58=0.42 | 0.84 | 0.84 | 1.00 | 51.50 | 0.42÷0.42=1.00 |
| 3....... | 8.0−7.60=0.40 | 1.24 | 1.26 | 0.98 | 77.25 | 0.40÷0.42=0.95 |

By determining the electrical resistance it was found that *Laminaria* is killed by exposure to 35° C. for 70 minutes. The respiration before and after such exposure was then determined. During the treatment at 35° C. the material was removed from the tubes and placed in a large volume of the sea water kept at 35° C. The results are given in table VII A. After the exposure to 35° C., the relative amount and rate of respiration had fallen considerably below the normal. This might be expected on the ground that oxidizing enzymes are injured or destroyed by heat.

TABLE V A

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 6 PERIODS (30.5 MIN. EACH) IN SEA WATER; AT END OF SECOND PERIOD MATERIAL DRIED IN CURRENT OF AIR IN SUN FOR 139 MINUTES; MATERIAL THEN PLACED IN SEA WATER AT 22° C. FOR 15 MINUTES BEFORE BEGINNING THIRD PERIOD.

| Period | Change in PH | Change in PH after drying = A | Change in PH before drying, calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Time (in. min.) of exposure after drying | Relative rate of respiration |
|---|---|---|---|---|---|---|---|
| 1 | 8.15−8.0 =0.15 | ...... | ...... | ...... | 30.5 | 0 | ........ |
| 2 | 8.15−8.0 =0.15 | ...... | ...... | ...... | 61.0 | 0 | ........ |
| 3 | 8.15−7.20=0.95 | 0.95 | 0.15 | 6.3 | 91.5 | 30.5 | 0.95÷0.15=6.3 |
| 4 | 8.15−7.70=0.45 | 1.40 | 0.30 | 4.6 | 122.0 | 61.0 | 0.45÷0.15=3.0 |
| 5 | 8.15−7.85=0.30 | 1.70 | 0.45 | 3.8 | 152.5 | 91.5 | 0.30÷0.15=2.0 |
| 6 | 8.15−8.00=0.15 | 1.85 | 0.60 | 3.1 | 183.0 | 122.0 | 0.15÷0.15=1.0 |

It is well known that severe injury causes a considerable rise in the respiration, and it seemed desirable to make such experiments with *Laminaria*. After the normal respiration of a piece of tissue had been determined, the material was removed from the tube and finely macerated (by means of the jagged end of a tube of Pyrex glass) on a piece of tested filter paper. The minced *Laminaria* was put back into the tube and rinsed 6–10 times with sea water until none of the liberated pigment could be distinguished in the sea water. Fresh sea water was then added and the respiration determined. The results are given in table VIII, the control data being given in table V. In table VIII it will be observed that the relative amount and relative rate of respiration are both

TABLE VI A

Change in PH value of sea water produced by respiration of *Laminaria* during 3 periods (23 min. each) in sea water; at end of second period material placed in running tap water for 19 hours; material then placed in sea water at 22° C. for 34 minutes before beginning third period

| Period | Change in PH | Change in PH after exposure to tap water = A | Change in PH before exposure to tap water, calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Time (in min.) of exposure after exposure to tap water | Relative rate of respiration |
|---|---|---|---|---|---|---|---|
| 1...... | 8.0−7.7=0.3 | ....... | ....... | ....... | 23 | 0 | .............. |
| 2...... | 8.0−7.7=0.3 | ....... | ....... | ....... | 46 | 0 | .............. |
| 3...... | 8.0−8.0=0.0 | 0 | 0.3 | 0 | 69 | 23 | 0.0÷0.3=0.0 |

TABLE VII

Control for VII A: 4 periods (25.5 min. each) in sea water; between second and third periods material kept in sea water at 16° C. for 70 minutes

| Period | Change in PH | Total change in PH = A | Change in PH calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Relative rate of respiration |
|---|---|---|---|---|---|---|
| 1....... | 8.0−7.57=0.43 | 0.43 | 0.425 | 1.01 | 25.5 | 0.43÷0.425=1.01 |
| 2....... | 8.0−7.58=0.42 | 0.85 | 0.85 | 1.00 | 51.0 | 0.42÷0.425=0.99 |
| 3....... | 8.0−7.58=0.42 | 1.27 | 1.275 | 1.00 | 76.5 | 0.42÷0.425=0.99 |
| 4....... | 8.0−7.58=0.42 | 1.69 | 1.70 | 0.99 | 102.0 | 0.42÷0.425=0.99 |

TABLE VII A

Change in PH value of sea water produced by respiration of *Laminaria* during 4 periods (28 min. each) in sea water; between second and third periods material placed in large volume of sea water and kept at 35° C. for 70 minutes

| Period | Change in PH | Change in PH after exposure following second period = A | Change in PH immediately after second period = B | Relative amount of respiration = A/B | Time in min. | Time (in min.) of exposure during last two periods | Relative rate of respiration |
|---|---|---|---|---|---|---|---|
| 1....... | 8.0−7.25=0.75 | ....... | ....... | ....... | 28 | ....... | ................ |
| 2....... | 8.0−7.28=0.72 | ....... | ....... | ....... | 56 | ....... | ................ |
| 3....... | 8.0−7.80=0.20 | 0.20 | 0.735 | 0.27 | 84 | 28 | 0.20÷0.75=0.26 |
| 4....... | 8.0−7.85=0.15 | 0.35 | 1.470 | 0.24 | 112 | 56 | 0.15÷0.75=0.20 |

more than doubled, but gradually decline. After 1 hour the relative rate of respiration was still above the normal. In this case the time of death could not be determined.

The experiments show that although the rate of respiration may be maintained for a time after death, it gradually falls off and eventually becomes very small. The question arises whether this falling off is due to exhaustion of the supply of oxidizable material or not. It is clear that when respiration has practically ceased there is a considerable amount of organic material left, but it is by no means certain that this material is such as to be easily oxidized by the ordinary processes which produce $CO_2$. On the

TABLE VIII

CHANGE IN PH VALUE OF SEA WATER PRODUCED BY RESPIRATION OF *Laminaria* DURING 5 PERIODS (31.25 MIN. EACH) IN SEA WATER; BETWEEN SECOND AND THIRD PERIODS MATERIAL FINELY MINCED

| Period | Change in PH | Change in PH after mincing = A | Change in PH before mincing, calculated from first 2 periods = B | Relative amount of respiration = A/B | Time in min. | Time (in min.) of exposure after mincing | Relative rate of respiration |
|---|---|---|---|---|---|---|---|
| 1....... | 8.1−7.70=0.40 | ....... | ....... | ....... | 31.25 | ....... | ................ |
| 2....... | 8.1−7.70=0.40 | ....... | ....... | ....... | 62.50 | ....... | ................ |
| 3....... | 8.1−7.05=1.05 | 1.05 | 0.40 | 2.6 | 93.75 | 31.25 | 1.05÷0.40=2.6 |
| 4....... | 8.1−7.65=0.45 | 1.50 | 0.80 | 1.9 | 125.00 | 62.50 | 0.45÷0.40=1.1 |
| 5....... | 8.1−7.90=0.20 | 1.70 | 1.20 | 1.4 | 156.25 | 93.75 | 0.20÷0.40=0.5 |

other hand, we must consider the possibility that the production of $CO_2$ falls off because the supply of oxidizing enzymes is used up. Various observers have found that these enzymes may be used up (or inactivated) during oxidation (1, 9). If the process of oxidation involves the cooperation (or successive action) of various enzymes the inactivation of any one of them might bring the whole process to a standstill.

WARBURG (21), as the result of extensive study, has come to the conclusion that the rate of oxidation depends on the amount of "structure" which the cell possesses. If the "structure" is partially or completely destroyed the oxidation diminishes in proportion, except in rare cases (as in the unfertilized sea urchin egg) where the oxidation is independent of structure. He states that the latter case disposes of the "reaction chamber" theory of cell structure, according to which the substances necessary for oxidation are separated by the semipermeable membranes of the cell in such a way as to regulate the speed of oxidation, for these substances can be completely mixed, as in the cytolysis of the unfertilized sea urchin egg, without any change in the rate of oxidation.

WARBURG's treatment of the "reaction chamber" hypothesis seems to rest upon a misunderstanding. It is quite possible that in the cytolysis of the sea urchin egg the "reaction chambers" are not destroyed, since each of the fine granules into which the egg is resolved by cytolysis may be such a "reaction chamber" surrounded by a semipermeable surface.[4] In case some or all of the reaction chambers are destroyed by the treatment, because they are larger or for any reason more sensitive to the treatment, a change in the rate of oxidation may be expected (either an increase or decrease, according to circumstances). WARBURG himself states that where an increase of chemical action results from the injury the "reaction chamber" hypothesis seems to be justified. This is precisely what the writer finds. Increase of oxidation as the result of injury (although not as the result of death) has previously been recorded by many observers (5).

The "reaction chamber" hypothesis has much in its favor. An especially good example is the bitter almond, which at once produces HCN upon injury. In this case the reacting substances are known and we cannot escape the conclusion that previous to injury they fail to react because they are kept apart by structures in the cell. In some cases the mingling of substances, owing to the breaking down of such separating structures, can distinctly be seen under the microscope. This is the case with the marine alga *Griffithsia*, as described by OSTERHOUT (14, 15). When cells of this alga are injured by poisons ($NH_4Cl$), or mechanically, or by cytolysis with dilute sea water, the chromatophores (which contain a soluble red pigment) become permeable and the pigment can be seen passing

[4] The existence of an actual membrane is unnecessary.

out into the surrounding cytoplasm. It would seem, therefore, that in the absence of a better explanation[5] the reaction chamber hypothesis might serve a useful purpose.

## Summary

The respiration of *Laminaria* after death may be considerably greater than in its normal condition. This is the case when it is killed by alcohol, acetone, formaldehyde, and ethyl bromide, as well as by drying and by other methods.

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[5] The explanation suggested by WARBURG (21), that the oxidizing substances are bound up by the structure, seems too vague for discussion.

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